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(54) Title: LEUKOCYTE REMOVAL METHOD AND FILTER UNIT FOR SAME

(57) Abstract

A method for removing leukocytes from a leukocyte-containing suspension comprising passing said suspension through a filter including a nitrocellulose membrane having a pore size of 5-15 μm.

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LEUKOCYTE REMOVAL METHOD AND FILTER UNIT FOR SAME

The present invention relates to a method for removing leukocytes from suspensions containing them and especially from blood-derived suspensions. The invention also relates to a filter unit which can be used in the above method.

In recent years it has become apparent that leukocytes in transfused blood are in most cases not only superfluous but often detrimental. Leukocytes in the blood have been found to cause non-hemolytic febrile reactions and alloimmunization as well as to harbor viruses.

Donated blood can be used without prior treatment ("whole blood"), or, more frequently, processed to produce a red cell or platelet concentrate. Various methods have been developed to remove leukocytes from these blood products, the most popular being filtration methods.

EP 155003 (to Asahi) and US 4925572 (to Pall) describe filtration methods using fibrous, non-woven media to capture the leukocytes as the blood suspension is passed through them. A leukocyte removal rate of over

98% can be obtained using these methods. The disadvantage of these methods is that the filter units used in them have to be composed of many layers of filter media in order to reduce leukocyte counts efficiently while at the same time providing a reasonable flow rate. This makes industrial assembly cumbersome and expensive.

An alternate method of leukocyte filtration has been described in Patent Application EP 406485 (to NPBI Nederlands). This method uses continuous porous membranes for the filtration process instead of fibers. One of the materials used for preparing the membranes described in the above application was cellulose acetate, which was found to give the best results. However, as can be seen in Fig. 9 of the above application, over 35% of the leukocytes remained in the blood filtrate, resulting in a leukocyte removal of less than 65% as compared to greater than 98% for conventional methods. The authors of the above application came to the conclusion that a series of stacked membranes having decreasing pore size in the blood flow direction ('asymmetric filter') showed a larger leukocyte removal capacity than membranes of uniform pore size. However, only a 25% leukocyte removal percentage was obtained with this 'asymmetric' filter, which contained a series of 8 membranes (Fig. 8). The filtration method described in the above application is therefore not useful for the purpose of leukocyte removal from blood products.

Other recent patents have described the use of polyvinylidene fluoride and polyvinyl formal membranes for use in leukocyte removal

(see e.g. WO 89/02304).

It is an object of the present invention to provide a method for removing leukocytes from a blood-derived suspension which is efficient, simple and inexpensive.

It is a further object of the invention to provide a filter unit for use in such a method.

In accordance with this invention there is thus provided a method for removing leukocytes from a leukocyte-containing suspension comprising passing the suspension through a filter including a nitrocellulose membrane having a pore size of 5-15 μm.

It has surprisingly been found that by using commercially available nitrocellulose membranes, it has been possible to obtain a leukocyte removal percentage of between 97-99% using no more than 2 layers (Note: in this application, a membrane is defined as a continuous, nonfibrous porous matrix, and a layer is defined as consisting of one or more identical filtering elements). This result is unexpected since nitrocellulose membranes are known to be strong binding matrices for proteins. Due to this property they are used extensively in diagnostic kits where protein probes (antigens or antibodies) are irreversibly bound to the membrane surface. It could have been expected that such membranes would clog very easily upon contact with blood due to the binding of blood proteins and corpuscles. It was therefore surprising to find that such membranes are very efficient leukofilters. The filtration is accomplished with a minimal loss of erythrocytes, and blood flow is equivalent to that of conventional fiber filters.

Without limiting the invention in any way, it is thought that the mechanism by which this separation works is probably related to a selective adsorption of leukocytes on the membrane surface. The separation cannot depend on size alone, as erythrocytes and many leukocyte types are of similar size. The invention, however, does not depend on any particular theory of separation.

The method of the invention can be carried out using a single nitrocellulose membrane. Alternatively, two nitrocellulose membranes can be used, or a non-woven or membrane prefilter overlying a nitrocellulose membrane. In a further embodiment of the invention, non-woven fiber sheets can be inserted between individual nitrocellulose membranes so as to act as spacers. Ordinarily, no more than two layers are necessary in order to obtain optimal results. The non-woven prefilter can be prepared from polymer fibers such as polyesters, polyurethane and polypropylene.

The pore size of commercial nitrocellulose membranes compatible with the method of the invention ranges from 5-15μm, preferably 8-12μm, and more preferably 8-10μm. Nitrocellulose membranes with pore sizes greater than 15μm are generally not available commercially. However, if such membranes become available, it may also be possible to use them in the invention. The pore size of the prefilter also ranges from 5-15μm.

Nitrocellulose membranes which have been chemically modified may also be used in the method of the invention. This chemical modification may be accomplished by performing a

surface grafting reaction in which the membrane is exposed to a suitable initiator while in contact with a monomer. The initiator may be chemical, or it may be gamma or U.V. radiation. Suitable monomers can be various acrylic monomers carrying functional groups such as hydroxy, carboxy or others.

In accordance with this invention there is also provided a filter unit having a filter for removing leukocytes from a leukocyte-containing suspension, the filter including a nitrocellulose membrane having a pore size of 5-15 μm.

Such a filter unit can be constructed, for example, from a conventional reusable filter holder. One or two layers according to the invention are placed in the filter holder which is tightly closed and connected to a reservoir containing a unit of blood or blood product. The blood or blood product is then forced through the filter unit by applying hydrostatic pressure. The layers can be easily replaced after one or more blood units have been filtered through them. Alternatively, the filter unit can be constructed from a disposable filter housing into which the layers are heat sealed, glued or clamped mechanically.

Various leukocyte-containing suspensions can be filtered using the method of the invention. These include whole blood, packed red blood cells and platelet concentrate.

The following examples come to further illustrate and describe the invention disclosed herein. The invention is not to be limited in scope by reason of any of the following examples.

Example 1

A 25mm diameter nitrocellulose membrane having a nominal pore size of 12μm was mounted in a holder of polysulfone. Both membrane and holder are products of Schleicher and Shuell, Germany. Whole blood that was stored for 7 days at 5°C. with citrate anticoagulant was filtered through the filter unit at a hydrostatic pressure of 0.08 atm. The effective filtration area was 3.2 cm^2 and the flow rate was 0.53 mL/min.

Results: When the number of cells in the filtrate was measured, the leukocyte count was found to be reduced from 5600 cells per μl in the original blood to 200 cells per μL, a reduction of 96.5%, while the erythrocyte count remained practically unchanged ($4.7x10^6$ to $4.6x10^6$ cells/μl).

Example 2

The procedure of Example 1 was repeated, except that an additional nitrocellulose membrane having a nominal pore size of 8μm was added beneath the first membrane. The filtration rate was 0.4 mL/min.

Results: The filtered blood was found to contain 50 leukocytes/mL (a 99.1% reduction) and $4.6x10^6$ erythrocytes/mL.

Example 3

The procedure of Example 2 was repeated, except that the two layers were a 8μm nitrocellulose membrane overlayed with a 5μm polyvinyl-chloride prefilter. 24hrs.-old blood was filtered at a rate of 0.5 mL/min.

Results: The leukocyte count of the filtered blood dropped from 10,100 cells/μl to 100 cells/μl (a 99% reduction) while the erythrocyte count slightly dropped from $3.9x10^6$

to $3.8x10^6$ cells/μl.

Example 4

The procedure of Example 3 was repeated, except that the 8μm nitrocellulose membrane was replaced with a 12μm membrane, and a red cell concentrate was used instead of whole blood. The red cell concentrate was 7 days old and stored at 5°C. The filtration rate was 2.0 mL/min.

Results: The leukocyte count dropped 97.3% from 7300 to 200 leukocytes/μl while the erythrocyte count dropped slightly from $8.3x10^6$ to $7.9x10^6$ cells/μl.

Example 5

The procedure of Example 3 was repeated, except that a non-woven polyurethane prefilter was placed on top of a 12 μm nitrocellulose membrane. A membrane holder made of Delrin (Gelman Scientific, U.S.A.) was used having an effective filtration area of 3.7 cm^2. 48hrs.-old whole blood was filtered at a rate of 2.1 mL/min.

Results: The leukocyte count was reduced from 7000 to 100 cells/μl (a 98.6% reduction) while the erythrocyte count remained substantially the same ($4.0x10^6$ to $3.9x10^6$ cells/μl).

Example 6

The procedure of Example 1 was repeated, except that a 8μm nitrocellulose membrane grafted with hydroxy-ethyl-metacrylate was used. The blood flow rate was 0.5 mL/min.

Results: The filtrate contained 50 leukocytes per μl (a 99.1% reduction) while the erythrocyte count remained almost unchanged ($4.5x10^6$, down from $4.6x10^6$).

Example 7

Three 47mm diameter nitrocellulose membranes of 12μm pore size were mounted in a polycarbonate holder resulting in a 12 cm^2 effective filtration area. The membranes were overlayed with a prefilter comprising four identical sheets of polyester fiber. A unit (290 ml) of packed red blood cells (PRC) containing citrate-phosphate-dextrose (C.P.D.) anticoagulant was prepared by adding 100 ml of SAG-M preservation solution to a final hematocrit of 60%. The filtration was performed at room temperature 6 hrs. after donation.

Results: The first 50 ml of PRC were filtered in 10 min. The leukocyte count of the filtered PRC was found to have dropped from 7400 cells/μL to 4 cells/μL.

Example 8

The procedure of Example 7 was repeated, except that a 20hrs.-old PRC unit was used. The prefilter comprised 8 identical sheets of non-woven polyester, each sheet having a thickness of approximately 0.5mm and a fiber diameter of 5-8μm. The first 70 ml were filtered in 15 min.

Results: The leukocyte count was found to be reduced from 13100 cells/μL to 16 cells/μL. No significant changes were observed in the hematocrit (49.4% vs. 50.5%) or hemoglobin concentration (17.0 gr% vs. 17.7 gr%) after filtration.

Example 9

The procedure of Example 8 was used with 6 hrs.-old PRC which had been stored at 20-22°C., and was found to have a 49% hematocrit after addition of SAG-M. The rate of filtration was 152 ml/20 min.

Results: The leukocyte count dropped from 5500 cells/μL to 30 cells/μL, while the hematocrit remained at 48-48.6%.

Example 10

The procedure of Example 8 was repeated with 7 days old PRC having a 62.1% hematocrit and 21.9 gr% hemoglobin concentration. 36 ml were filtered in 20 min.

Results: The Leukocyte count was reduced from 4500 cells/μL to about 2 cells/μL. The post-filtration hematocrit was 60.3-61.3% and the hemoglobin concentration was 21.0-21.1 gr%.

Example 11

In this Example, the filter comprised a prefilter composed of 5 identical sheets of non-woven fiber as in Example 8, and 3 nitrocellulose membranes as in Example 7 interspersed by 3 spacer sheets of the same non-woven fiber as in the prefilter. All other parameters were as in Example 8.

Results: A 6% improvement in the flowrate was achieved without affecting the parameters of the filtered PRC. When the filter was used in the procedure of Example 9, a 19% improvement in the flowrate was obtained.

Example 12

The procedure of Example 11 was repeated in a larger diameter system having an effective filtration area of 50 cm^2. 360 ml of PRC treated as in Example 7 were filtered in less than 10 min. The pre-filtration values of the PRC were: leukocyte count - 8300 cells/μL; hematocrit - 47.9%; hemoglobin - 17.2 gr%; red blood cell count - 5.99×10^6 cells/μL.

Results: The post-filtration values of the PRC were respectively: 3 cells/μL; 47.2-

48%; 17.0-17.1 gr%; 5.90-5.93x10^6 cells/μL.

Example 13

The configuration of Example 1 was used with a platelet concentrate containing 1.1x10^6 plt/μL and 800 leukocytes/μL. The filtration rate was 4 ml/2.5 min. After the nitrocellulose membrane surface was modified, e.g. by coating it with a polymer containing hydroxyl and sulfonic groups, the flowrate was increased to 4 ml/1.8 min.

Results: The leukocyte count was reduced to 200 cells/μL and the platelet count ranged between 0.34-1.04x10^6.

C L A I M S

1. A method for removing leukocytes from a leukocyte-containing suspension comprising passing said suspension through a filter including a nitrocellulose membrane having a pore size of 5-15 μm.

2. A method according to claim 1, wherein said filter consists of a single layer.

3. A method according to claim 1, wherein said filter comprises two or more overlying layers, at least one of said layers comprising said nitrocellulose membrane.

4. A method according to claim 3, wherein said filter consists of only two said layers.

5. A method according to either of claims 3 or 4, wherein each of said layers comprises a said nitrocellulose membrane.

6. A method according to either of claims 3 or 4, wherein one of said layers, to constitute a pre-filter, comprises a non-woven polymer fiber.

7. A method according to any of claims 1-6, wherein said filter comprises a layer consisting of a plurality of nitrocellulose membranes interspersed by non-woven polymer fiber spacers.

8. A method according to either of claims 6 or 7, wherein said non-woven polymer fiber is chosen from the group comprising polyester, polyurethane and polypropylene.

9. A method according to claim 1, wherein said membrane has a pore size of 8-12μm.

10. A method according to claim 1, wherein said nitrocellulose membrane is chemically modified by a surface grafting reaction while in contact with a monomer.

11. A method according to claim 10,

wherein said monomer comprises an acrylic monomer.

12. A filter unit having a filter for removing leukocytes from a leukocyte-containing suspension, said filter including a nitrocellulose membrane having a pore size of 5-15 μm.

13. A filter unit according to claim 12, wherein said filter consists of a single layer.

14. A filter unit according to claim 12, wherein said filter comprises two or more overlying layers, at least one of which layers comprises said nitrocellulose membrane.

15. A filter unit according to claim 14, wherein said filter consists of only two said layers.

16. A filter unit according to either of claims 14 or 15, wherein each of said layers comprises a said nitrocellulose membrane.

17. A filter unit according to either of claims 14 or 15, wherein one of said layers, to constitute a pre-filter, is a non-woven polymer fiber.

18. A filter unit according to any of claims 12-17, comprising a layer consisting of a plurality of nitrocellulose membranes interspersed by non-woven polymer fiber spacers.

19. A filter unit according to either of claims 17 or 18, wherein said non-woven polymer fiber is chosen from the group comprising polyester, polyurethane and polypropylene.

20. A filter unit according to claim 12, wherein said membrane has a pore size of 8-12μm.

21. A filter unit according to claim 12, wherein said nitrocellulose membrane is chemically modified by a surface grafting reaction while in contact with a monomer.

22. A filter unit according to claim 21, wherein said monomer comprises an acrylic monomer.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/01413

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :B01D 37/00, 39/00, 71/10
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 210/645, 650, 651, 767, 483, 490, 500.21, 500.29, 504, 506, 508; 536/31, 127

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS
search terms: leukocyte(s), filter(s), nitrocellulose, membrane(s)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US, A, 4,734,192 (CHAMPION ET.AL.) 29 March 1988, see entire document	12, 14-15, 20 --- 13, 16
Y	US, A, 4,925,572 (PALL) 15 March 1990, see entire document	17, 19
Y	US, A, 5,137,633 (WANG) 11 August 1992, see entire document	21, 22
Y,P	US, A, 5,185,127 (VONK) 09 February 1993, see entire document	18

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER:
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210/645, 650, 651, 767, 483, 490, 500.21, 500.29, 504, 506, 508; 536/31, 127